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Effects of Drilling Fluids on Embryo Development

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EFFECTS OF DRILLING FLUIDS ON EMBRYO DEVELOPMENT

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16. ABSTRACT

Toxicity of used drilling fluids to embryo development was investigated to ascertain the limits of safe usage of these fluids in marine environments. Embryos used as test systems were of the teleost, Fundulus heteroclitus, and four echinoderms Echinarachnius parma, Strongylocentrotus purpuratus, Lytechinus pictus, and Lytechinus variegatus. The drilling fluids tested came ffrom various sources; 24 different samples were evaluated. In addition, several commercial drilling fluid components were examined in the test systems incluidng a synthetic reference mud. Studies demonstrated that no single drilling fluid is "typical" and that the quantitative effects of embryos vary considerably from one fluid to another. Some drilling fluids are quite toxic to one or more of the embryo systems, requiring dilutions of over 10^5 to become "safe." Others are relatively innocuous, requiring very little dilution to achieve a concentration in which embryo development can proceed normaly. Also, the effects on development show variation from one fluid to another, thus indicating that a variety of components or compounds are responsible for toxic manifestations.

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FOREWORD

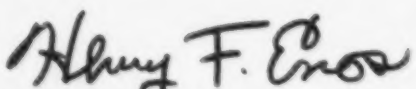
The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The EPA Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

the effects of toxic organic pollutants on individual species and communities of organisms;

the effects of toxic organics on ecosystem processes and components;

the significance of chemical carcinogens in the estuarine and marine environments.

This study specifically investigates the toxicity of spent or used drilling fluids from off-shore wells in the Gulf of Mexico to a teleost fish and four echinoderms. These data can be used to evaluate the impact of drilling operations for oil exploration on these marine organisms.



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ABSTRACT

The objective of this research program was to determine the toxicity of seawater soluble components of used drilling fluids to embryo development in order to help ascertain the limits of safe usage of these fluids in marine environments.

Embryos used as test systems in these assays were of the teleost, Fundulus heteroclitus and of four echinoderms, Echinarachnius parma, Strongylocentrotus purpuratus, Lytechinus pictus, and Lytechinus variegatus. Gametes were collected in season, combined for fertilization and the resultant embryos were reared in various concentrations of the drilling fluids. Any departure from normal developmental patterns was noted and the embryotoxicity for each fluid was determined.

The drilling fluids tested came from various sources; 24 different samples were evaluated including a synthetic reference mud. In addition, several commercial drilling fluid components were examined in the test systems.

It is very clear from these studies that no single drilling fluid is "typical", that the quantitative effects on embryos vary considerably from one fluid to another. Some of the drilling fluids are fairly toxic to one or more of the embryo systems, requiring dilutions of over 10^5 to become "safe". Others are relatively innocuous, requiring very little dilution to achieve a concentration in which embryo development can proceed normally. Also, the effects on development show variation from one fluid to another. While some affect cardiac function in the fish embryo, others prevent hatching. In the echinoderm embryo, cleavage is prevented by some drilling fluids while others inhibit development at the events of gastrulation. This suggests that there are a variety of components or compounds responsible for toxic manifestations. Identification of the components responsible for toxicity must await chemical analysis of these fluids.

This report was submitted in fulfillment of the requirements of Assistance Agreement No. CR807102 granted by the U.S. Environmental Protection Agency. This report covers the period May 15, 1980 to August 20, 1982, and the work was completed as of September 15, 1982.

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INTRODUCTION

As a result of petroleum drilling offshore, large quantities of drilling fluids (muds) are introduced into the marine environment. These drilling fluids are aqueous suspensions of a variety of components pumped down the center of the drill bit. The composition of such fluids varies with the needs of the drilling operation, such as lubrication, cooling, prevention of intrusion of seawater into the bore hole, antibacterial action, suspension of drill cuttings, and capture of H_2S. The drilling fluid may be partially or wholly discharged into the surrounding waters during the drilling program or especially at its completion. This discharge can amount to significantly large quantities (e.g., 2,000 tons per hole) in the vicinity of a drilling platform.

The impact of drilling fluids on marine and estuarine environments has been largely due to the paucity of toxicological studies. Recently studies on a variety of biological parameters affected by drilling fluids have been reported by Derby and Atema (1981), Tagatz et al. (1978, 1979, 1980), Miller et al. (1980), Neff et al. (1980, 1981), Crawford and Gates (1981), Rubenstein et al. (1980), Gerber et al. (1980), Conklin et al. (1980), Carr et al. (1980), Tornberg et al. (1980), Houghton et al. (1980), Thompson and Bright (1980), Krone and Biggs (1980), and Hudson and Robbin (1980). In each case, a drilling fluid was found to have an adverse effect on a biological model system.

The studies reported here were carried out to examine in some detail the effect of several representative drilling fluids on fertilization and embryo

development. As model systems, embryos of an estuarine teleost, Fundulus heteroclitus, were chosen as well as embryos of several echinoderms. These models represent a variety of habitats and embryo types which could be found in the vicinity of petroleum drilling operations. In addition, each of these species represents an important member of the food chain. Conditions which might interfere with their reproductive capacity would have effects more far reaching than simply on the species alone.

MATERIALS AND METHODS

Drilling Fluids:

Drilling fluids tested were supplied by the U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida. They may be divided into three groups according to the locale or agency of collection.

Group I: collected in Alabama from a Mobile Bay drilling rig in 1979. These are identified by the date the sample was taken.

March 12
April 24
May 29
June 26
October 11

Group II: collected in Florida from the Jay Field. These samples are identified by the following numbers designated by the EPA.

1-1	2-21
1-2	3-3
2-17	3-16
2-18	4-20
2-20	5-1

Group III: collected for EPA by Petroleum Equipment Suppliers Association from various sources. These samples are identified by the following numbers designated by the EPA.

MIBLKA51
SV-76
AN31
P1
P2
P3
P4
P5

In addition, a synthetic drilling fluid was tested. This is a reference drilling mud prepared by the National Bureau of Standards. The dry components are mixed with water and NaOH by specified formulation.

All stock drilling fluid samples were stored in plastic bottles at 4°C. Diluted test samples were prepared shortly before use and kept at the temperature of the test system.

Drilling Fluid Components:

Samples of typical commercial components of drilling fluids were supplied by the U.S. EPA, Environmental Research Laboratory, Gulf Breeze, Florida. The following components were tested, either individually or in various combinations: Soltex, Barite, Imco PolyRx, Imco VC-10, Imco RD-111, Imco SulfX II, Milgel, and Lubrikleen.

Standardization of Drilling Fluid Test Solutions:

All drilling fluids tested were standardized according to dry weight. Since many of the stock samples exhibited precipitation or settling, resuspension by stirring was often the most time-consuming task. From the resuspended homogenous slurry, an aliquot was diluted tenfold. 5.0 ml portions were removed from the diluted stock and transferred to tared 10 ml beakers. These were dried overnight at 110°C and then reweighed. This determination

was run four times for each stock mud. The dry weights of the drilling fluids tested are given in Table 1. The stock drilling fluids were diluted with seawater to a concentration of 10 parts per thousand (ppt) and then stirred for 1 hour at room temperature. Upon dilution with seawater, much additional precipitation occurred and therefore after stirring the suspension was passed through Whatman #1 filter paper. This stock solution, termed 10 ppt, was diluted with seawater to make up the 1 ppt, 100 parts per million (ppm), 10 ppm, and 1 ppm test solutions. Thus, the test solutions contained the seawater-soluble components of the drilling fluids.

TABLE 1. DRILLING FLUID DRY WEIGHTS

Drilling Fluid		mg/ml (ppt)	Dilution at 10 ppt
Group I	3/12	174	1:17.4
	4/24	294	1:29.4
	5/29	220	1:22.0
	6/29	262	1:26.2
	10/11	418	1:41.8
Group II	1-1	135	1:13.5
	1-2	135	1:13.5
	2-17	207	1:20.7
	2-18	204	1:20.4
	2-20	210	1:21.0
	2-21	210	1:21.0
	3-3	201	1:20.1
	3-16	190	1:19.0
	4-20	163	1:16.3
	5-1	223	1:22.3
Group III	MI	367	1:36.7
	SV	1132	1:113.2
	AN	650	1:65.0
	P1	1064	1:106.4
	P2	1101	1:110.1
	P3	1331	1:113.9
	P4	1139	1:130.0
	P5	1300	1:130.0
Synthetic		426	1:42.6

In some cases, the pH had to be adjusted to fall within the range determined to have no effect on the tests (6.0 to 8.5). The pH values of the 10 ppt solutions before adjustments are given in Table 2. Adjustments were made by adding HCl or NaOH.

TABLE 2. pH OF DRILLING FLUID SOLUTIONS (10 ppt) BEFORE ADJUSTMENT

Drilling Fluid		pH	Drilling Fluid		pH
Group I	3/12	11.45	Group III	MI	8.55
	4/24	9.50		SV	8.25
	5/29	11.55		AN	8.40
	6/26	11.30		P1	7.80
	10/11	10.90		P2	8.25
				P3	7.75
Group II	1-1	7.80		P4	7.30
	1-2	7.45		P5	7.75
	2-17	8.10			
	2-18	8.25	Components		
	2-20	8.40		VC-10	3.80
	2-21	8.35		Barite	7.30
	3-3	8.05		RD-111	5.05
	3-16	8.05		Sulfx II	8.20
	4-20	8.05		Soltex	7.35
	5-1	8.50		Poly Rx	5.90
				Milgel	7.90
Synthetic		7.40			

Fundulus Development Test

Embryos of Fundulus heteroclitus were used as the model for teleost development studies. The adult fish were obtained from estuarine waters of Frenchman Bay, Maine, and kept in floating live cars in seawater. Gametes were obtained and fertilized, and embryos cultured according to descriptions found in New (1966), Crawford et al. (1973) and Crawford and Gates (1981). Eggs were stripped from females, and fertilization was initiated by adding a sperm suspension obtained by mincing dissected testes. Filtered seawater kept at 18°C was the normal incubation medium. Developmental stage references used were those of Oppenheimer (1937).

For these studies, embryos were placed in the drilling fluid solutions 1 minute after fertilization and maintained at those concentrations for the duration of their development. The incubation dishes contained 10 ml of medium, changed daily, and from 25 to 100 embryos. The particularly susceptible events to environmental toxins observed with the dissecting microscope included early cleavage, blastulation, gastrulation, formation of the body axis, appearance of somites, yolk and body pigmentation, heart development and circulation, eye pigmentation, formation of organs and various fins, jaw and body movements, and hatching. At 18°C, the time from fertilization to hatching is approximately 25 days.

Sand Dollar Development Test:

Embryos of the sand dollar Echinarachnius parma were used as one model for echinoderm development studies. Adults were collected in Frenchman Bay, Maine and were kept in running seawater tanks at 16°C. Gametes were obtained by coelomic injection of about 1 ml of 0.5 M KCl, as described by Hinegardner (1967). Fertilization was begun by adding a few drops of a 1% sperm suspension to a suspension of eggs in about 100 ml of filtered seawater. The normal incubation medium was filtered seawater kept at 16°C. The progress of development was observed with a compound microscope, particularly noting any effects on early cleavage, blastulation, hatching of the blastula to a spinning form, gastrulation, development of the prism and formation of the pluteus as described by Karnofsky and Simmel (1963). When noting the progress of pluteus development, attention was directed to motility and to the symmetry and lengths of the "arms". Approximately 10,000 embryos were placed in 30 ml of each incubation medium 10 to 15 minutes after fertilization and remained in these solutions for the duration of the experiment (4 days).

Sea Urchin Development Test:

Embryos of several sea urchins were also used as models of echinoderm development studies. During the autumn, Lytechinus variegatus (obtained from Gulf Specimen Co., Panacea, Florida) and Lytechinus pictus (obtained from Pacific Biomarine Co., Venice, California) were appropriate for obtaining ripe gametes. During the winter and spring, Strongylocentrotus purpuratus (also obtained from Pacific Biomarine) is an excellent choice for gametes. The sea urchins were kept in aquaria at 12°C in simulated sea water (Instant Ocean). However, it was found that the sea urchin embryo development was more successful in natural sea water. Gametes were obtained, fertilization was begun, and observations were made as described above for sand dollars.

Echinoderm Egg Fertilization Tests:

Gametes were preincubated for 15 minutes in the drilling fluid solutions prior to mixing. Fertilization was initiated by adding 5 drops of the 1% sperm suspension to 20 ml of egg suspension. Within two minutes, the fertilized eggs may be identified microscopically as those with raised fertilization membranes. In each experiment, percentage fertilization was determined by observing 100 eggs.

RESULTS

Fundulus Development:

Drilling fluids of Group I and Group III and drilling fluid components were tested for their effects on Fundulus development. The results of these studies showed the considerable variability of the fluids tested.

Group I:

Embryos in 10 ppt of the October 11 sample showed developmental delay during early stages of gastrulation. These embryos continued development, although slowly, until late stages of organogenesis at which they were arrested and died. Their appearance and movements were normal, except for a reduction in pigmentation. The June 26 sample produced another effect at the 10 ppt and 1 ppt levels. At the onset of organogenesis development was delayed and the rate of heart beat was reduced as shown in Table 3. The 10 ppt embryos all died without further development while the 1 ppt group seemed to recover by the time hatching stages were reached.

TABLE 3. EFFECT OF JUNE 26 DRILLING FLUID ON CARDIAC CONTRACTIONS IN FUNDULUS EMBRYOS

Drilling fluid in medium	Heart beats per minute[a]					
	14[b]	16	18	21	25	32
0(controls)	89	110	105	141	141	140
1 ppm	89	110	107	136	140	140
10 ppm	90	106	111	134	140	138
100 ppm	96	105	107	133	140	140
1 ppt	77	87	90	122	102	135
10 ppt	32	28	26	all dead	------	

[a] heart beats per minute determined by 30 second counts on at least 10 embryos.

[b] days post-fertilization.

Other effects of these fluids were not seen until hatching (Table 4). In some cases, the hatching process itself was disturbed, leading to a cessation of development at that point. In other media, hatched fry showed abnormal behavior, being unable to coordinate swimming movements. Their compromised swimming often meant that they could not swim up; i.e., they

TABLE 4. EFFECTS OF GROUP I DRILLING FLUIDS ON HATCHING AND FRY OF FUNDULUS

Drilling Fluid	Concentration	No. Embryos	% Hatched[a]	% Fry Alive[a]	Condition of Fry[a]
3/12	0	99	80	96	Normal
	10 ppm	96	91	95	Normal
	100 ppm	92	82	94	Normal
	1 ppt	100	71	97	Normal
	10 ppt	99	21	77	Can't right selves
4/24	0	99	92	90	Normal
	10 ppm	103	86	96	Normal
	100 ppm	92	87	90	Normal
	1 ppt	104	76	96	Normal
	10 ppt	103	50	60	Can't right selves
5/29	0	99	92	90	Normal
	10 ppm	99	88	97	Normal
	100 ppm	99	92	87	Normal
	1 ppt	98	80	94	Lethargic Compromised swim.
	10 ppt	100	69	87	Severely uncoord.
6/26	0	100	85	95	Normal
	10 ppm	100	83	95	Normal
	100 ppm	100	79	99	Normal
	1 ppt	100	86	93	Compromised swim.
	10 ppt	100	0	--	--
10/11	0	93	78	91	Normal
	10 ppm	99	63	98	Normal
	100 ppm	34	88	97	Normal
	1 ppt	92	38	100	Normal
	10 ppt	91	0	--	--

[a]At 30 days post fertilization.

could not raise themselves from the bottom of the incubation dish. In other cases, the fry rested on their sides, unable to right themselves. It is assumed that in all instances of abnormal swimming ability, the fry could not survive, being unable to escape predation or to obtain food.

The March 3 sample prevented most hatching at the 10 ppt concentration and those fry which emerged could not right themselves. The April 24 and May 29 samples had some effect on hatching, and at high concentrations the fry were affected. Greatest toxicity in this group was shown by the June 26 and October 11 drilling fluids which prevented all hatching at 10 ppt and affected fry at high concentrations.

Components:

Several drilling fluid components were tested for their effects on Fundulus development and, as with the fluids themselves, the results were quite varied according to type of substance. Both VC-10 and PolyRx (lignosulfonates) had pronounced effects on heart beats at concentrations of 10 ppt. With both of these components, heart rates were slightly diminished during organogenesis. By day 30, those embryos in VC-10 had heart rates of 31% of normal while those in PolyRx had rates 60% of normal. The data are very similar to those shown in Table 3 for the June 26 drilling fluid. All of these affected embryos died before hatching could occur.

The other major effect of these components on Fundulus development was on hatching and health of fry. Only Barite had no effect at every concentration. The other components disturbed the hatching process as shown in Table 5. At 1 ppt VC-10, Sulfx II, and RD-111 significantly reduced the percentage of hatching. The fry which hatched from 1 ppt PolyRx were very lethargic,

TABLE 5. EFFECTS OF DRILLING FLUID COMPONENTS ON HATCHING AND FRY OF FUNDULUS

Component	Concentration	No. Embryos	% Hatched[a]	% Fry Alive[a]	Condition of Fry[a]
VC-10	0	68	84	98	Normal
	10 ppm	66	78	100	Normal
	100 ppm	51	84	98	Normal
	1 ppt	68	56	97	Lethargic
	10 ppt	41	0[b]	--	--
Barite	0	67	85	98	Normal
	10 ppm	40	70	100	Normal
	100 ppm	53	85	98	Normal
	1 ppt	65	65	95	Normal
	10 ppt	93	88	96	Normal
Soltex	0	44	89	100	Normal
	1 ppm	23	78	100	Normal
	10 ppm	21	76	100	Normal
	100 ppm	29	59	100	Normal
	1 ppt	28	68	26	Lethargic
Sulfx II	0	44	89	97	Normal
	1 ppm	25	84	95	Normal
	10 ppm	28	82	100	Normal
	100 ppm	27	59	100	Normal
	1 ppt	24	21	100	Normal
PolyRx	0	29	59	94	Normal
	10 ppm	20	85	88	Normal
	100 ppm	24	71	71	Normal
	1 ppt	31	70	90	Lethargic
	10 ppt	27	0[c]	--	--
RD-111	0	29	66	100	Normal
	1 ppm	30	80	100	Normal
	10 ppm	41	71	97	Normal
	100 ppm	43	67	90	Normal
	1 ppt	28	32	0	All dead

[a]At 30 days post-fertilization.
[b]Heart beat first showed reduction to 78% at 9 days post-fertilization and was 31% of controls at 30 days post-fertilization.
[c]Heart beat first showed reduction to 81% at 15 days post-fertilization and was 60% of controls at 30 days post-fertilization.

TABLE 6. EFFECTS OF GROUP III DRILLING FLUIDS ON HATCHING AND FRY OF FUNDULUS

Drilling Fluid	Concentration	No. Embryos	% Hatched[a]	% Fry Alive[a]	Condition of Fry[a]
MIBLKA51	0	136	93	93	Normal
	1 ppm	39	87	97	Normal
	10 ppm	92	73	97	Normal
	100 ppm	65	69	93	Normal
	1 ppt	44	91	95	Normal
	10 ppt	74	45	97	Severely uncoord.
SV-76	0	174	80	96	Normal
	1 ppm	61	82	92	Normal
	10 ppm	61	80	96	Normal
	100 ppm	43	91	95	Normal
	1 ppt	77	66	96	Lethargic
	10 ppt	57	84	96	Severely uncoord.
AN31	0	136	93	93	Normal
	1 ppm	61	82	100	Normal
	10 ppm	49	75	93	Normal
	100 ppm	67	75	98	Normal
	1 ppt	59	88	96	Normal
	10 ppt	67	64	67	Severely uncoord.
Synthetic	0	174	80	96	Normal
	1 ppm	64	86	96	Normal
	10 ppm	49	94	89	Normal
	100 ppm	49	88	100	Normal
	1 ppt	58	91	85	Lethargic
	10 ppt	59	31[b]	72	Severely uncoord.
P1	0	43	91	90	Normal
	1 ppm	40	85	100	Normal
	10 ppm	29	83	100	Normal
	100 ppm	30	70	100	Normal
	1 ppt	27	93	92	Can't right selves
	10 ppt	25	4[c]	0	All dead
P2	0	43	91	90	Normal
	1 ppm	24	76	100	Normal
	10 ppm	22	100	95	Normal
	100ppm	28	93	96	Normal
	1 ppt	34	91	94	Lethargic
	10 ppt	33	48	75	Severely uncoord.

(continued)

TABLE 6. (continued)

Drilling Fluid	Concentration	No. Embryos	% Hatched[a]	% Fry Alive[a]	Condition of Fry[a]
P3	0	43	91	90	Normal
	1 ppm	19	95	89	Normal
	10 ppm	22	86	95	Normal
	100 ppm	19	79	93	Normal
	1 ppt	27	85	96	Normal
	10 ppt	46	52	96	Severely uncoord.
P4	0	110	80	89	Normal
	1 ppm	46	85	95	Normal
	10 ppm	50	72	86	Normal
	100 ppm	62	61	92	Normal
	1 ppt	64	78	92	Lethargic
	10 ppt	72	39	89	Severely uncoord.
P5	0	110	80	89	Normal
	1 ppm	45	87	87	Normal
	10 ppm	43	95	95	Normal
	100 ppm	32	91	100	Normal
	1 ppt	40	95	92	Lethargic
	10 ppt	45	71	88	Severely uncoord.

[a]At 30 days post-fertilization.

[b]Heart beats first showed reduction to 77% at 16 days post-fertilization and were 65% of controls at 28 days post-fertilization.

[c]Heart beats first showed reduction to 79% at 7 days post-fertilization and were 59% of controls at 27 days post-fertilization.

certainly unable to escape from predators and probably compromised in ability to catch food.

Group III:

Effects of the Group III drilling fluids on Fundulus development and hatching are shown in Table 6. Heart beat rates were affected by the 10 ppt concentration of synthetic mud and the P1 sample. Hatching was significantly affected only at the 10 ppt level and this was observed in the case of MI, AN, Synthetic, P1, P2, P3, and P4 fluids. The most sensitive effect seen was on the fry. Many of the fluids disturbed the normal patterns of behavior of fry at 1 ppt. These fry were lethargic and unable to swim upward to the surface. Those which were severely uncoordinated could barely move themselves off the bottom, and their swimming motions were random and uncontrolled.

Additional Tests with Fundulus:

a) Incubation beginning at late stages. Normal embryos at stages 28-29 (20 days post-fertilization) were placed in 10 ppt of the Group I drilling muds and observed for 21 days. The results are presented in Table 7. Hatching was compromised in the June 26 and October 11 fluids and the fry which did emerge could not swim. Although considerable hatching occurred with the March 12 fluid, the fry were unable to swim to the surface. The other fluids had no profound effect.

b) Incubation of Fry in drilling fluids. Normal fry were placed in 10 ppt of the Group I drilling fluids one day after hatching. Those in the June 26 and October 11 fluids did not survive beyond 6 days. By 10 days, no effects were noted using the other fluids.

c) Reversibility. Embryos were removed from 10 ppt solutions of Group I drilling fluids 1.5 days and 7 days post-fertilization (gastrula

and early organogenesis respectively). In all cases, recovery was complete.

d) Delayed onset of incubation. Embryos began their incubation in drilling fluids of Group I at fertilization, at gastrulation (1.5 days post fertilization), and at keel formation (3 days post fertilization). In each case, the effects on development and hatching were the same. That is, the effects on Fundulus development were not dependent on interference with some very early events of embryogenesis.

TABLE 7. EFFECT OF GROUP I DRILLING FLUIDS ON FUNDULUS DEVELOPMENT, INCUBATION BEGINNING AT STAGE 28-29.

Drilling Fluid (10 ppt)	No. Beginning Incubation at Stage 28-29	No. Hatched in 21 days	Condition of fry
3/12	20	15	Cannot swim off bottom
4/24	20	15	Normal
5/29	20	18	Normal
6/26	20	8	Cannot swim
10/11	20	5	Cannot swim
Controls	20	18	Normal

Fertilization Effects:

Effects of the drilling fluids on fertilization were observed using gametes from several different echinoderms. Table 8 shows the data from a typical experiment, in this case run on the Group I, October 11 drilling fluid. It should be noted that pretreatment of sperm with the drilling fluid has no effect (there are a few exceptions); the adverse effects on fertilization are due to interference with egg function.

Table 9 is a summary of fertilization studies on all fluids and components, using four different echinoderms as models. This table shows data for those concentrations which affected fertilization. Note that for the

most part, dilution to 100 ppm allowed normal fertilization. However, some of the components were toxic even at a concentration of 1 ppm.

TABLE 8. EFFECT OF OCTOBER 11 DRILLING FLUID ON FERTILIZATION OF SAND DOLLAR EGGS

Drilling fluid treatment medium	Sperm-Egg Treatment Combinations	% Fertilization
10 ppm	Normal sperm + Normal eggs	98
	Treated sperm + Normal eggs	99
	Normal sperm + Treated eggs	99
	Treated sperm + Treated eggs	100
100 ppm	Normal sperm + Normal eggs	99
	Treated sperm + Normal eggs	99
	Normal sperm + Treated eggs	100
	Treated sperm + Treated eggs	96
1 ppt	Normal sperm + Normal eggs	98
	Treated sperm + Normal eggs	99
	Normal sperm + Treated eggs	0
	Treated sperm + Treated eggs	0
10 ppt	Normal sperm + Normal eggs	99
	Treated sperm + Normal eggs	100
	Normal sperm + Treated eggs	0
	Treated sperm + Treated eggs	0

No attempt was made to analyze these data statistically. Generally the effects are all-or-none. Where the effects seem to be partial, in most cases, only one or two experiments had been run. The number of instances where partial effects were averaged by several experiments is less than ten. In those cases, calculation of the standard deviation of the mean demonstrated that the data were significant. The reproducibility of these experiments is excellent if one is careful to use only those results from gametes whose control rate of fertilization is 95% or greater.

Echinoderm Development:

The results of all drilling fluids and components tested on four

TABLE 9. EFFECTS OF DRILLING FLUIDS AND COMPONENTS ON ECHINODERM FERTILIZATION

Drilling Fluid	Concentration[a]	% Fertilization of treated eggs[b]			
		E.parma	S.purpuratus	L.pictus	L.variegatus
Group I					
3/12	10 ppt	72 (5)			
4/24	10 ppt	8 (3)			
5/29	10 ppt	7 (5)			
6/26	10 ppt	1 (4)			
10/11	10 ppt	0 (3)			
	1 ppt	1 (3)			
Group II					
1-1	10 ppt	96 (1)	93 (2)	14 (1)	100 (1)
1-2	10 ppt	38 (1)	87 (2)	94 (1)	
2-17	10 ppt	90 (1)	48 (2)	0 (1)	0 (1)
2-18	10 ppt	60 (2)	95 (3)	2 (1)	1 (1)
2-20	10 ppt	96 (1)	2 (1)	5 (1)	3 (1)
	1 ppt	100 (1)	100 (1)	77 (1)	25 (1)
2-21	10 ppt	60 (1)	52 (2)	2 (1)	1 (1)
	1 ppt	100 (1)	100 (2)	15 (1)	23 (1)
3-3	10 ppt	45 (2)	45 (3)	1 (1)	0 (1)
	1 ppt	99 (2)	91 (3)	13 (1)	2 (1)
3-16	10 ppt	26 (1)	27 (3)	0 (2)	0 (1)
	1 ppt	100 (1)	98 (3)	57 (2)	63 (1)
4-20	10 ppt	1 (1)	9 (1)	0 (2)	1 (1)
	1 ppt	100 (1)	100 (1)	34 (2)	23 (1)
5-1	10 ppt	17 (1)	0 (3)	0 (2)	17 (1)
	1 ppt	80 (1)	84 (3)	32 (2)	80 (1)
Group III					
SV	10 ppt	0 (1)	0 (5)		
	1 ppt	94 (1)	0 (5)		
	100 ppm	100 (1)	84 (5)		
AN	10 ppt	0 (1)	12 (5)		
	1 ppt	100 (1)	76 (5)		
MI	10 ppt	100 (1)	100 (4)		
P1	10 ppt	0 (1)	9 (1)		
P2	10 ppt	0 (1)	0 (1)		
	1 ppt	100 (1)	69 (1)		
P3	10 ppt	0 (1)	66 (1)		
P4	10 ppt	0 (1)	1 (1)		
	1 ppt	95 (1)	7 (1)		
P5	10 ppt	0 (1)	10 (2)		
Synthetic	10 ppt	0 (1)	0 (5)		
	1 ppt	21 (1)	0 (5)		
	100 ppm	100 (1)	54 (5)		

(continued)

TABLE 9. (continued)

Drilling Fluid	Concentration[a]	% Fertilization of treated eggs[b] E.parma	S.purpuratus	L.pictus	L.variegatus
Components					
VC-10	1 ppt	3 (1)	0 (5)	0 (4)	
	100 ppm	84 (1)	13 (5)	0 (4)	
	10 ppm		100 (5)	31 (4)	
	1 ppm			73 (4)	
PolyRx	100 ppm	0 (1)	0 (2)		
	10 ppm	99 (1)	94 (2)	3 (2)	
	1 ppm			62 (2)	
RD-111	1 ppt	48 (1)	0 (4)		
	100 ppm	94 (1)	69 (4)	2 (1)	
	10 ppm		99 (4)	44 (1)	
	1 ppm			80 (1)	
Sulfx II	1 ppt	100 (2)	72 (4)		
	100 ppm		67 (4)		
	10 ppm		93 (4)	3 (1)	
Soltex	100 ppm	4 (1)	3 (3)	3 (1)	
Barite	10 ppt	99 (1)	99 (4)		
Milgel	10 ppt		91 (3)		
Lubrikleen	100 ppm		0 (3)		
	10 ppm		47 (3)		

[a] At all lower concentrations fertilization was not affected.
[b] Figures are all normalized to percentage of control fertilization. Number in parentheses is number of experiments run.

different echinoderm embryos are given in Table 10. Only the concentrations which adversely affected development are listed, showing the stage of development disturbed. Adverse effect is defined as that observed in a population of embryos showing anything less than the control rate of development. In a good or useful experiment the development of controls is quite synchronous; if it is not, the experiment is discarded. The stage of arrest or abnormality is quite distinct for a given set of conditions and the appearance of the affected embryos is similar. For example, if abnormal gastrula development occurs due to exogastrulation, these deformed types will dominate the field.

It should be noted that there is a remarkable similarity among species regarding these effects. Ordinarily, L. pictus is somewhat more sensitive than the other organisms. Toxicity varies considerably from one fluid to another; however, only one of them (SV) shows toxic manifestations if diluted to 10 ppm.

In other types of experiments using both E. parma and S. purpuratus, the initiation time of incubation in the drilling fluids was varied. The fluids of Group I were introduced at 10 minutes post-fertilization, at the morula stage, at the blastula, and at the gastrula. In all cases, the developmental effects were similar. A delay of initiation time caused the stage of arrested development to be more advanced.

TABLE 10. EFFECTS OF DRILLING FLUIDS AND COMPONENTS ON ECHINODERM EMBRYO DEVELOPMENT

Drilling Fluid	Concentration[a]	Stage of arrest or abnormality			
		E. parma	S. purpuratus	L. pictus	L. variegatus
Group I					
3/12	10 ppt	Blastula	Mid gastrula		Late gastrula
	1 ppt	No effect	Early prism		No effect
4/24	10 ppt	Blastula	Late gastrula		Late gastrula
	1 ppt	Early pluteus	Early prism		Early prism
5/29	10 ppt	Blastula	Late gastrula		Late gastrula
	1 ppt	Early pluteus	Early prism		No effect
6/26	10 ppt	Blastula	Early gastrula		Early gastrula
	1 ppt	Early pluteus	Mid gastrula		Late gastrula
10/11	10 ppt	1st cleavage	1st cleavage		1st cleavage
	1 ppt	Early pluteus	Early gastrula		Late gastrula
	100 ppm	No effect	Early prism		Early prism
Group II					
1-1	10 ppt	No effect	No effect	Prism	Late gastrula
	1 ppt	No effect	No effect	No effect	Early pluteus
1-2	10 ppt	No effect	No effect	Early pluteus	Mid pluteus
2-17	10 ppt	Early pluteus	Late gastrula	Prism	Early pluteus
	1 ppt	Late pluteus	Prism	Prism	Mid pluteus
	100 ppm	Late pluteus	Prism	Early pluteus	No effect
2-18	10 ppt	Early pluteus	Late gastrula	Late gastrula	Early pluteus
	1 ppt	Mid pluteus	Prism	Prism	No effect
	100 ppm	No effect	Prism	No effect	No effect
2-20	10 ppt	No effect	Late gastrula		Early pluteus
	1 ppt	No effect	Prism		Mid pluteus
	100 ppm	No effect	Prism		No effect
2-21	10 ppt	Early pluteus	Late gastrula	Late gastrula	Mid pluteus
	1 ppt	Mid pluteus	Prism	Late gastrula	No effect
	100 ppm	No effect	Prism	No effect	No effect

(continued)

TABLE 10. (continued)

Drilling Fluid	Concentration[a]	Stage of arrest or abnormality			
		E. parma	S purpuratus	L. pictus	L.variegatus
Group II					
3-3	10 ppt	Prism	Late gastrula	Prism	Mid pluteus
	1 ppt	No effect	Prism	Prism	No effect
	100 ppm	No effect	Prism	Early pluteus	No effect
3-16	10 ppt	Prism	Late gastrula	Mid gastrula	Early pluteus
	1 ppt	No effect	Prism	Late gastrula	Mid pluteus
	100 ppm	No effect	Prism	No effect	No effect
4-20	10 ppt	Early gastrula	Late gastrula	Mid gastrula	Late prism
	1 ppt	No effect	Prism	Late gastrula	Mid pluteus
	100 ppm	No effect	Prism	No effect	No effect
5-1	10 ppt	Prism	Late gastrula	Mid gastrula	Early pluteus
	1 ppt	No effect	Late gastrula	Late gastrula	Mid pluteus
	100 ppm	No effect	Prism	Prism	No effect
Group III					
SV	10 ppt	Gastrula	Blastula		
	1 ppt	Early pluteus	Prism		
	100 ppm	No effect	Prism		
	10 ppm	No effect	Prism		
AN	10 ppt	Gastrula	Prism		
	1 ppt	Mid pluteus	Prism		
	100 ppm	No effect	Prism		
MI	10 ppt	Gastrula	Gastrula		
	1 ppt	Late pluteus	Prism		
P1	10 ppt	Morula			
	1 ppt	Early gastrula			
	100 ppm	Early pluteus			
P2	10 ppt	Early gastrula			
	1 ppt	Early pluteus			

(continued)

TABLE 10. (continued)

Drilling Fluid	Concentration[a]	Stage of arrest or abnormality			
		E. parma	S. purpuratus	L. pictus	L. variegatus
Group III					
P3	10 ppt	Late gastrula			
	1 ppt	Early pluteus			
	100 ppm	Mid pluteus			
P4	10 ppt	Late gastrula			
	1 ppt	Early pluteus			
P5	10 ppt	Early gastrula			
	1 ppt	Mid pluteus			
Synthetic	10 ppt	Blastula	Gastrula		
	1 ppt	Early pluteus	Prism		
	100 ppm	Mid pluteus	Prism		
	10 ppm	No effect	Prism		
Components					
VC-10	10 ppt	1st cleavage	1st cleavage	1st cleavage	1st cleavage
	1 ppt	Late blastula	Gastrula	Mid gastrula	Gastrula
	100 ppm	Gastrula	Late gastrula	Late gastrula	Prism
	10 ppm	No effect	Prism	Late gastrula	No effect
Poly Rx	10 ppt	Early blastula	Morula	1st cleavage	1st cleavage
	1 ppt	Late blastula	Blastula	Late blastula	Late blastula
	100 ppm	Gastrula	Gastrula	Gastrula	Late gastrula
	10 ppm	Late pluteus	Late gastrula	Late gastrula	No effect
RD-111	10 ppt	1st cleavage	1st cleavage		
	1 ppt	Gastrula	Late gastrula		Cleavage
	100 ppm	Mid pluteus			Mid pluteus
Milgel	10 ppt		No effect		No effect
Sulfx II	1 ppt	Gastrula	Late blastula	Late gastrula	Late gastrula
	100 ppm	Mid pluteus	Gastrula	Late gastrula	Prism
	10 ppm	No effect	Gastrula	Late gastrula	Prism

(continued)

TABLE 10. (continued)

Drilling Fluid	Concentration[a]	Stage of arrest or abnormality			
		E. parma	S. purpuratus	L. pictus	L. variegatus
Components					
Soltex	1 ppt	Blastula	Late blastula		Blastula
	100 ppm	Prism	Gastrula		Prism
	10 ppm	No effect	Prism		No effect
Barite	10 ppt	No effect	Prism		
Lubrikleen	100 ppm		Late blastula		
	10 ppm		Late prism		

[a] At all lower concentrations, development was not affected.

DISCUSSION

It is clear from these studies that seawater-soluble components of drilling fluids are toxic to Fundulus and echinoderm embryos and that echinoderm eggs are rendered infertile by exposure to these fluids. Table 11 represents a "worst case" survey from the data of this report, showing the "safe" concentration of drilling fluids in the most sensitive test. Also, the required dilution of the drilling fluid in question to reach that "safe" concentration is shown. "Safe" in this case means a concentration having no adverse effect on the system.

By reviewing the data in Table 11 one must be impressed by the large variation in toxicity among the different drilling fluids. Safe concentrations may be achieved by dilution of 135-fold in one case or 1,132,000-fold in the other extreme. Clearly, no single drilling fluid is "typical"; indeed, the synthetic fluid is among the most toxic tested.

Of course, it is of great interest to determine the nature of the active components found in the most toxic fluids. To that end, some chemical analyses have been made on many of the muds tested (Shokes, 1982). Total quantities in whole drilling fluids were determined for Al, Ba, Cd, Ca, Cr, Cu, Fe, Pb, Sr, Zn, aliphatics, and aromatics. When matched with toxicity studies, it is difficult to make any clear correlations with the concentrations of any one component. The closest possibilities are with Cr and Zn, whose concentrations are shown in Table 12. The most toxic fluids, SV, synthetic, and 10/11, have elevated Cr levels. Cr concentration may be a reflection of the content of lignosulfonates present in the drilling fluid. The significance of this

TABLE 11. HIGHEST DRILLING FLUID CONCENTRATIONS HAVING NO EFFECT ON EITHER FUNDULUS OR ECHINODERM EMBRYOGENESIS

Drilling Fluid	"Safe" concentration in most sensitive test	
	Concentration (ppm)[a]	Dilution[b]
Group I: 3/12	100	1,740
4/24	100	2,940
5/29	100	2,200
6/26	100	2,620
10/11	10	41,800
Group II: 1-1	100	1,350
1-2	1,000	135
2-17	10	20,700
2-18	10	20,400
2-20	10	21,000
2-21	10	21,000
3-3	10	20,100
3-16	10	19,000
4-20	10	16,300
5-1	10	22,300
Group III: MI	100	3,670
SV	1	1,132,000
AN	10	65,000
P1	10	106,400
P2	100	11,010
P3	10	133,100
P4	100	11,390
P5	100	13,000
Synthetic	1	426,000
Components		
VC-10	<1	
Barite	1000	
RD-111	<1	
Sulfx II	1	
Soltex	1	
PolyRx	<1	
Milgel	>10,000	
Lubrikleen	1	

[a] Refer to description of preparation of drilling fluid solutions in text. Safe level refers to a concentration of 10% of that having an adverse effect in the most sensitive test system.

[b] Dilution of original drilling fluid suspension to achieve the safe concentration.

lies in that these are among the most toxic components tested. However, P1 and P3 are among the most toxic fluids and there is nothing remarkable about their Cr levels. Furthermore, the Cr level in AN is about one-third that of 6/26 yet its toxicity is much greater. Also, if Cr alone was the only toxic component, the 6/26 and 5/29 fluids would be more toxic than SV. Zn content also offers both positive and negative correlations.

TABLE 12. ANALYSIS OF Cr AND Zn IN WHOLE DRILLING FLUIDS[a]

Drilling Fluid		Cr ug/g	Zn ug/g
Group I:	3/12	35.4	62.4
	4/24	662	55.7
	5/29	1810	98.6
	6/26	2400	163
	10/11	5420	606
Group III:	MI	337.2	161.3
	SV	1345.3	495.3
	AN	748.9	232.9
	P1	814.4	409.5
	P2	482.5	2064
	P3	458.9	438.5
	P4	532.3	1384.3
	P5	187.0	175.0
Synthetic		2733.6	29.8

[a]Data provided for EPA by R. Shokes, Science Applications, Inc.

Attempts to correlate combinations of any two components with toxicity have been unprofitable. All this might be anticipated for such complex mixtures; it would be reasonable to expect that no component is the sole source

of toxicity and indeed in many instances combinations of components may produce effects in excess of the sum of their individual effects.

A further point should be made regarding these analyses. They were performed on the whole drilling fluid, not on the seawater-soluble fraction. Therefore the data do not bear directly on an analysis of components responsible for the toxic effects shown in this report.

Another factor which must be determined before environmental significance can be assigned these data is the actual dilution which occurs at a drilling rig and the surrounding area. What are the stabilizing and dispersing factors? How long can a concentration gradient be maintained? Are there any factors which cause concentration of some components of a drilling fluid? The data presented here show some biological effects to be expected if certain concentrations of the drilling fluids are maintained for the requisite time period.

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